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Original Article

Prevalence of neutralizing antibodies against AAV8, AAV9, and AAV843 in a Chinese population

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Abstract: Adeno-associated virus (AAV) is a tiny, non-enveloped, and single-stranded DNA virus that requires helper viruses to promote efficient replication. Recombinant AAV has been extensively studied as the most potential vector for gene therapy. However, the presence of AAV neutralizing antibodies is one of the key factors impairing the efficiency of transduction. Two natural serotypes, AAV8 and AAV9, have lower neutralizing antibody titers. They have been considered as candidate serotypes for gene therapy in European and Japanese populations. However, the prevalence of neutralizing antibodies of the above two AAV serotypes in the Chinese population remains unclear. Recently, neutralizing antibodies (NAbs) against two natural AAVs (AAV8 and AAV9) and one novel serotype (AAV843) that were derived from DNA shuffling were investigated in the serum of 154 Chinese people. Results showed that the prevalence of AAV8, AAV9, and AAV843 NAbs was 38.3%, 35.7% and 25.5%, respectively. In addition, although the prevalence of NAbs in these three AAV serotypes was increased with age, in AAV9 and AAV843 it was more robustly increased with age (≤10, 11-20, 21-30, & 31-40 vs ≥41, $P<0.001$). Ninety-one people (59%) had none of these three serotypes, but 37 people (24%) were positive for all three AAV serotypes. In the 154 people, only 6 samples were positive for single AAV8 NAb, while 4 samples were positive for single AAV9 Nabs. However, single AAV843 NAbs was not detected in any. Comparing titers in people with two or more NAbs, AAV8 Nab titer was higher than that of AAV9 in 90.7% of the samples ($P<0.05$). AAV843 Nab titer was always the lowest. In summary, prevalence of AAV8 and AAV9 was low in the Chinese population. AAV843 NAb was almost non-existent. AAV8, AAV9, and AAV843 can be used as candidate serotypes for gene therapy in Chinese populations, but AAV843 is the most optimal. The current study provides an important logical basis for development of gene therapy drugs using these serotypes.

Keywords: Neutralizing antibodies, adeno-associated virus, prevalence, gene therapy

Introduction

Gene therapy is a robust biomedical technology for correction and altering of gene expression for disease treatment. It has been used to treat single-gene genetic diseases and multisystem complex diseases, such as malignant tumors, infectious diseases, cardiovascular diseases, autoimmune diseases, and metabolic diseases. Recently, recombined adeno-associated virus (rAAV), as one of the biosafety and highly-effective gene deliverers, has been used in gene therapy. Moreover, rAAV is an artificially modified single-stranded DNA virus. It has the ability to infect non-dividing cells. In nature, AAV has been detected in many human tissues, but has not been associated with diseases [1-3]. Although AAV has the ability to replicate and integrate into the host chromatin after infections, it is a low risk for teratogenicity and carcinogenesis. However, immunogenicity of AAV in the body impairs the efficiency of genic transduction [4-6]. A previous study showed that serotype-specific neutralization antibody interfered with AAV bind cell surface, limiting AAV transduction [7, 8]. AAV NAbs in people that were pre-infected by AAV significantly reduced efficiency of rAAV gene therapy because of host memory immune response. Recombined AAV transduction could be sensitively inhibited although low NAbs titers in serum. Previously, AAV expressing recombined FIX was completely

Table 1. Three-plasmid system for packaging adeno-associated virus

Target gene plasmid	Capsid plasmid	Helper plasmid
AAV-Lxp3.3-Gluc	AAV2 Rep/AAV8 Cap	pHelper
	AAV2 Rep/AAV9 Cap	
	AAV2 Rep/AAV843 Cap	

neutralized when the NAbs titers were as low as 1:5 [9, 10]. In this population, individuals with positive NAbs were 30% to 40%. These people might have decreased AAV delivered gene expression, even immunotoxicity. Therefore, these people are not suitable for AAV gene therapy [11].

In European populations, prevalence rates of AAV1 and AAV2 neutralizing antibodies are 59% and 50.5%. AAV8 and AAV9 are 19% and 33.5%, respectively [12]. AAV8 and AAV9 neutralizing antibodies not only have low prevalence, but also showed lower neutralizing antibody titers in 70-100% positive individuals. In AAV gene therapy, AAV8 penetrates the vascular endothelium barrier to deliver therapeutic genes into the liver, heart, and skeletal muscle more effectively [6, 13, 14]. AAV9 has shown strong transduction ability to neurons in the brain [15]. In addition, several non-natural novel AAV serotypes have been invented for avoiding NAbs neutralizing therapeutic rAAV, an effectively deliver for gene therapy. AAV843, a novel variant from AAV2 that was developed by DNA shuffling, has shown a high affinity to the liver. It could be used in liver-targeted gene therapy [16]. Previously, several studies have revealed the prevalence of AAV8 and AAV9 NAbs in countries around the world [12, 17]. However, analysis of the prevalence of these two AAVs in China has not been widely reported. In this study, Gauss luciferase was used to detect prevalence rates of AAV8, AAV9, and AAV843 neutralizing antibodies, examining the cross-reactivity between them. The current study will provide a theoretical basis for the clinical application of AAV8, AAV9, and AAV843 in China.

Materials and methods

Blood collection

This epidemiological study was approved by the relevant Ethical Review Boards of the institutions and medical facilities that participated in this study. No therapeutic interventions were performed in this study. Blood samples were collected from 154 Chinese projects, ranging in age from 2 to 66, by Institute of Hematology & Blood Diseases, Tianjin City, China. The samples were grouped by age, as follows: 43 subjects less than 10 years old; 23 subjects from 11 to 20 years old; 30 subjects from 21 to 30 years old; 24 subjects from 31 years old to 40 years old; 34 subjects over 41 years old. Informed consent was obtained from all study participants. The samples were given new identification numbers upon blood collection with individual information removed, except for birth year. Serum from blood samples was stored at -80°C.

Generation of recombinant AAV

AAV8, AAV9, and AAV843 were generated by transient transfection of three AAV plasmids into HEK293 cells (**Table 1**). The cells were harvested 72 hours after transfection. They were then treated with 25 units/mL of benzonase nuclease (Sigma Aldrich, Louis, MO). AAV particles were purified by odixanol stepwise gradient ultracentrifugation (Oppiprep, Sigma Aldrich). They were then were concentrated in phosphate buffered saline to a final volume of 1 mL using an Amicon Ultra 10K centrifugal filter (Millipore, Bedford, MA). Titers of rAAV were detected by real-time PCR. After virus titration, rAAV was aliquoted and stored at -80°C.

Neutralizing antibody detection

Huh7 cells were divided into 5×10^4 cells per well into 96-well plates before virus infection. The next day, in 96-well plates, diluted 12 µl subject serum was placed into 108 µl serum-free DMEM, then continuously diluted 2 times into 5 concentration gradients. Finally, dilution ratios were 1, 1:2, 1:4, 1:8, 1:16, 1:32. Next, 60 µl 2×10^{10} VG/ml luciferase-expressing AAV particles were added to each well for one hour at 37°C [18-20]. After incubation, the Huh7 cell culture medium was replaced with 100 µl above serum-DMEM mixture and vehicle. After 48 hours of infection, researchers mixed 20 µl supernatant with 50 µl Gaussian luciferase substrate mixture (Cat# E3300, New England BioLab) and luciferase activity was measured using a Biotek automatic plate reader (Biotek Synergy Neo2). If the activity of Gauss lucifer-

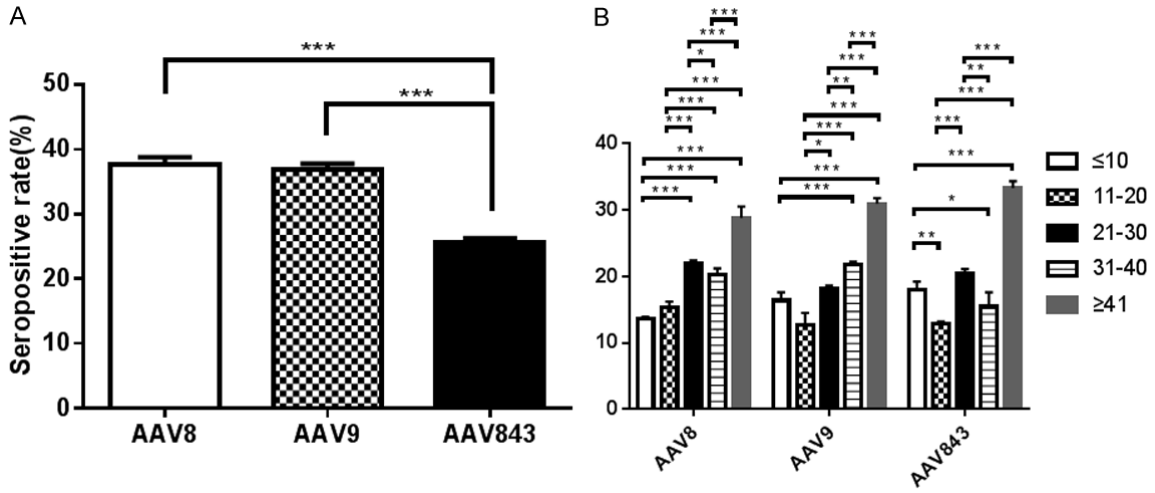

Figure 1. Prevalence of neutralizing antibodies against AAV serotypes 8, 9, and 843. A. Prevalence of neutralizing antibodies against AAV serotypes 8, 9, and 843; B. Neutralizing antibody seropositivity against AAV serotypes 8, 9, and 843 sub-grouped by ages for the Chinese.

ase was reduced to less than 50% of the negative control (Commercial mouse serum, Catalog number S-3509 Sigma), the dilution ratio of the serum was defined as the neutralizing antibody titer [21]. Seropositivity titer for neutralizing antibodies was defined in various ways, according to previous studies, suggesting that a titer between 1:5-1:10 is sufficient to neutralize any serotype, usually with an upper limit of 1:20 [21-23].

Statistical analysis

Chi-square tests and Fisher's exact tests were used for statistical analysis via SPSS 22.0. *P*-values <0.05 indicate statistical significance. Statistical analyses were performed using Prism 6.0 (GraphPad Software).

Results

Prevalence of neutralizing antibodies against AAV8, AAV9, and AAV843 increased with age of the subjects

Detection of activity of Gauss luciferase showed that 59 subjects were AAV8 NAb (38.3%) positive and 55 subjects were AAV9 NAb (35.7%) positive, while only 39 subjects were AAV843 NAb (25.5%) positive (**Figure 1A**). The prevalence of AAV843 NAb was significantly lower than that of AAV8 and AAV9 (**Figure 1A**, AAV8 vs AAV9, P>0.05; AAV8 vs AAV843, P<0.01; AAV9 vs AAV843, P<0.01).

In 59 AAV8 NAb positive subjects, 8 subjects (18.6%) were under 10 years old, 9 subjects (39.1%) were 11 to 20 years old, 13 subjects (43.3%) were 21 to 30 years old, 12 subjects (50.0%) were 31 to 40 years old, and 17 subjects (50.0%) were over 41 years old (Table S1). In 55 AAV9 NAb positive subjects, 9 subjects (20.9%) were under 10 years old, 7 subjects (30.4%) were 11 to 20 years old, 10 subjects (33.3%) were 21 to 30 years old, 12 subjects (50.0%) were 31 to 40 years old, and 17 subjects (50.0%) were over 41 years old. In 39 AAV843 NAb positive subjects, the number of subjects under 10 years old, from 11 to 20 years old, from 21 to 30 years old, from 31 to 40, and over 41 years old were 7 (16.3%), 5 (21.7%), 8 (26.7%) 6 (25.0%), and 13 (38.2%), respectively (**Figure 1B**).

In addition, thirty-seven subjects were positive for all detected AAV Nabs. The number of subjects under 10 years old, from 11 to 20 years old, from 21 to 30 years old, from 31 to 40 years old, and over 41 years old were 7 (18.9%), 5 (13.5%), 7 (18.9%), 6 (16.2%), and 12 subjects (32.4%), respectively. In these subjects, comparing the NAbs titer, results showed that AAV8 NAb titer in 4 subjects was significantly higher than the other two serotypes (P<0.05). The AAV9 NAb titer in 2 subjects was significantly higher than the other two serotypes (P<0.05). The AAV843 NAb titer was the lowest in all triple-positive subjects. In addition, there

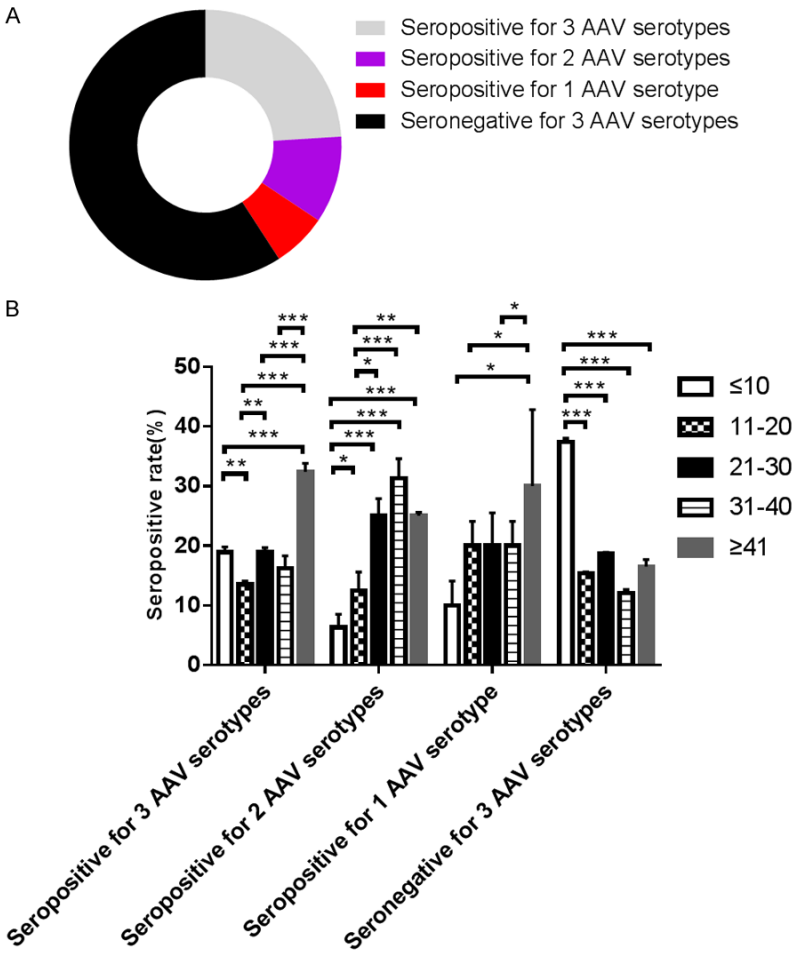

Figure 2. Neutralizing antibodies against multiple AAV serotypes. A. The proportion of neutralizing antibodies against multiple AAV serotypes; B. Neutralizing antibodies against multiple AAV serotypes sub-grouped by ages for the Chinese.

were 16 subjects with two AAV NAbs. The number of subjects under 10 years old, from 11 to 20 years old, from 21 to 30 years old, from 31 to 40 years old, and over 41 years old were 1 (6.3%), 2 (12.5%), 4 (25.0%), 5 (31.3%), and 4 (25%), respectively. Ten subjects were positive for a single AAV serotype. One individual (10.0%) was under 10 years old, 2 individuals (20.0%) were 11 to 20 years old, 2 individuals (20.0%) were 21 to 30 years old, 2 individuals (20.0%) were 31-40 years old, and 3 individuals (30.0%) were over 41 years old. The remaining 91 subjects had no detected AAV Nabs. Their age distribution was 34 subjects (37.4%) under 10 years old, 14 subjects (15.4%) from 11 to 20 years old, 17 subjects (18.7%) from 21 to 30 years old, 11 subjects (12.1%) from 31 to 40 years old, and 15 subjects (16.5%) were over 41 years old (Table S2). In summary, prevalence rates of AAV8 and AAV9 NAbs were higher than AAV843 Nab rates (**Figure 2**). It was observed that the proportion of individuals seropositive for neutralizing antibodies against each AAV serotype tested increased with age (**Figure 2**).

Neutralizing antibody seropositivity against AAV8, AAV9, and AAV843 in subjects in each age stage

In the youngest subject group, 8 subjects (13.6%) were AAV8 NAb positive and 9 subjects (16.4%) were AAV9 NAb positive, while 7 subjects (17.9%) were AAV843 NAb positive. With increases in age, the number of subjects with positive AAV8 and AAV9 NAbs increased significantly (**Figure 3A**). In the 11 to 20 years old group, 9 subjects (15.3%) were AAV8 NAb positive and 7 subjects (12.7%) were AAV9 NAb positive, while only 5 subjects (12.8%) were AAV843 NAb positive (**Figure 3A**, AAV8 vs AAV843, p<0.01; AAV9 vs AAV843, P<0.01). In the 21 to 30 years old group, 13 subjects (22.0%) were AAV8 NAb positive, 10 subjects (18.2%) were AAV9 NAb positive, and 8 subjects (20.5%) were AAV843 NAb positive (**Figure 3A**, AAV8 vs AAV843, P<0.001; AAV9 vs AAV843, P<0.001; AAV8 vs AAV9, P<0.001). In the 31 to 40 years old group, 12 subjects (20.3%) were AAV8 NAb positive, 12 subjects (21.8%) were AAV9 NAb positive, and 6 subjects were AAV843 NAb (15.4%) positive (**Figure 3A**, AAV8 vs AAV843, P<0.01; AAV9 vs AAV843, P<0.01). In the over 41 years old group, 17 subjects (28.8%) were AAV8 NAb positive, 17 subjects (30.9%) were AAV9 NAb positive, and 13 subjects were AAV843 NAb (33.3%) positive (**Figure 3A**, AAV8 vs AAV843, P<0.01; AAV9 vs AAV843, P<0.01). The above results showed that, except for subjects under 10 years old, the prevalence

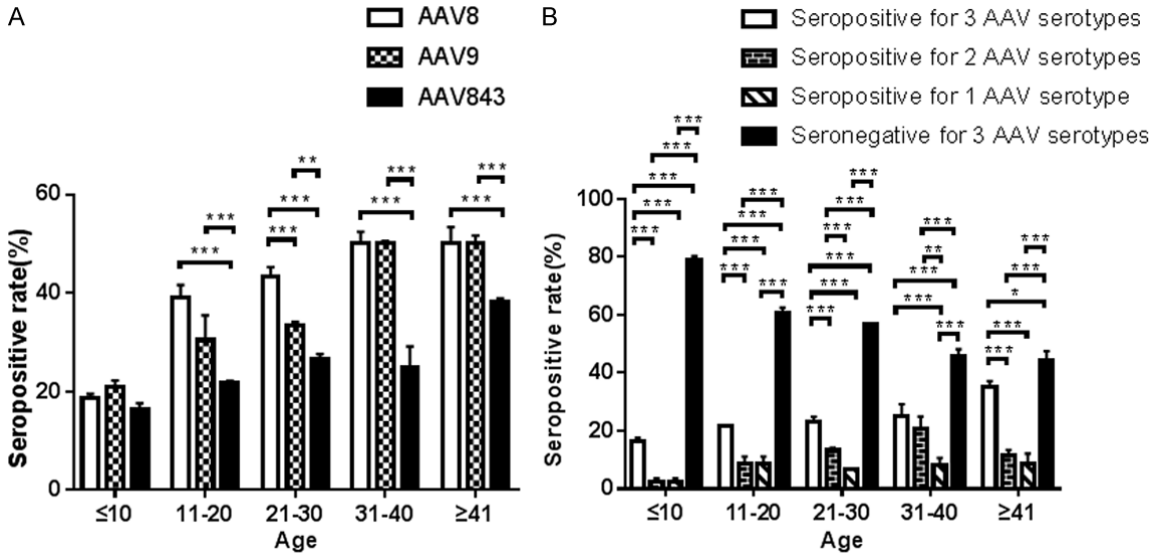

Figure 3. Neutralizing antibody seropositivity against AAV8, AAV9, and AAV843 in subjects in the same age group. A. Neutralizing antibody seropositivity against AAV serotypes 8, 9, and 843 in subjects in the same age group for Chinese; B. Neutralizing antibodies against multiple AAV serotypes in subjects in the same age group. Chinese subjects that were seropositive for all AAV serotypes (black bar) and seronegative for all AAV serotypes (white bar) in the same age group.

Table 2. Co-prevalence of NAbs to AAV8, AAV9, and AAV843

	Vector	AAV8	AAV9	AAV843
Positive sera	AAV8	59 (100%)	51 (92.7%)	39 (100%)
	AAV9	51 (86.4%)	55 (100%)	37 (94.9%)
	AAV843	39 (66.1%)	37 (72.5%)	39 (100%)

of the neutralizing antibodies to AAV8 and AAV9 in all other age groups was significantly higher than that of AAV843 (Table S3).

In the under 10 years old group, 7 subjects (16.3%) were all three AAV NAbs positive, 1 subject (2.3%) was AAV8 and AAV9 NAbs positive, 1 subject (2.3%) was AAV9 NAb positive, and 34 subjects (79.1%) were negative for all three AAV NAbs. In the 11 to 20 years old group, 5 subjects (21.7%) were all three AAV NAbs positive, 2 subjects (8.7%) were AAV8 and AAV9 NAbs positive, 2 subjects (8.7%) were only single-positive for AAV8 NAb, and 14 subjects (60.9%) were negative for all three AAV NAbs. In the 21 to 30 years old group, 7 subjects (23.3%) were all three AAV NAbs positive and 4 subjects (13.3%) were AAV NAbs dual-positive. Only 2 subjects (6.7%) were single-seropositive for AAV8 and AAV9, respectively. The remaining 17 subjects (56.7%) were negative for three AAV NAbs. In the 31 to 40 years old group, 6 subjects (25.0%) were all three AAV NAbs positive, 5 subjects (20.8%) were AAV NAbs dual-positive and 2 subjects (8.3%) were AAV NAbs single-positive , while 11 subjects (45.8%) were seronegative for all three AAV NAbs. In the over 41 years old group, 12 subjects (35.3%) were all three AAV NAbs positive, 4 subjects (11.8%) were AAV NAbs dual-positive, and 3 subjects (8.8%) were AAV NAbs single-positive, while 15 subjects (44.1%) were seronegative for all three AAV NAbs.

Present results revealed that the number of subjects with three AAV NAbs was significantly higher than that with 1 or 2 type of Nabs (**Figure 3B**). The proportion of subjects seropositive for all three AAV NAbs increased with age, while the proportion of negative subjects decreased significantly (Table S4). In this study, all AAV843 NAb positive subjects were almost AAV8 and AAV9 NAbs positive (100% vs 94.9%, P>0.05; **Table 1**). However, more than 90% of subjects were AAV8 and AAV9 or AAV8 and AAV843 NAbs dual-positive. AAV8 NAb titer was higher than AAV9 or AAV843. AAV9 NAb titer was also higher than AAV843 NAb titer (**Table 2**). Results demonstrated that the level of neutralizing activity directed against AAV843 was lower

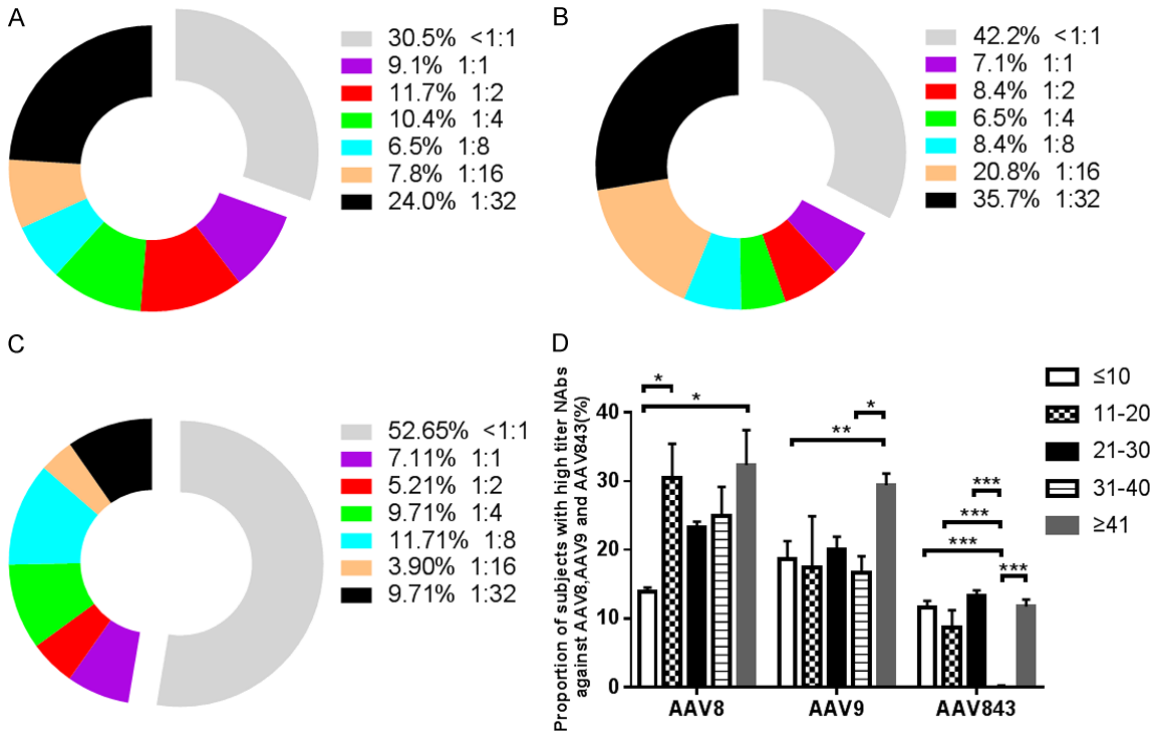

Figure 4. Neutralizing antibody titers against AAV8, AAV9, and AAV843. A. Titers of neutralizing antibodies against AAV8; B. Titers of neutralizing antibodies against AAV9; C. Titers of neutralizing antibodies against AAV843; D. Seroprevalence of neutralizing antibodies at high titers (≥1:32) against AAV8, 9, and 843.

than the level of neutralizing activity directed against AAV8 and AAV9 (in most samples).

Titers of AAV8, AAV9, and AAV843 NAbs increased with age

Neutralizing antibody titers against AAV8, AAV9, and AAV843 were quantified and the ranges of neutralizing antibody titers in age groups were analyzed (**Figure 4**). AAV8 NAb titer in 47 subjects (30.5%) was less than 1:1, in 14 subjects (9.1%) was 1:1, in 18 subjects (11.7%) was 1:2, in 16 subjects (10.4%) was 1:4, in 10 subjects (6.5%) was 1:8, in 12 subjects (7.8%) was 1:16, and in 37 subjects (24%) was 1:32 (**Figure 4A**). AAV9 NAb titer in 65 subjects (42.2%) was less than 1:1, in 11 subjects (7.1%) was 1:1, in 13 subjects (8.4%) was 1:2, in 10 subjects (6.5%) was 1:4, in 13 subjects (8.4%) was 1:8, in 10 subjects (20.8%) was 1:16, and in 32 subjects (35.7%) was 1:32 (**Figure 4B**). AAV843 NAb titer in 81 subjects (52.6%) was less than 1:1, in 11 subjects (7.1%) was 1:1, in 8 subjects (5.2%) was 1:2, in 15 subjects (9.7%) was 1:4, in 18 subjects (11.7%) was 1:8, in 6 subjects (3.9%) was 1:16, and in 15 subjects (9.7%) was 1:32 (**Figure 4C**).

In 37 subjects with 1:32 AAV8 NAb titer, 6 subjects (14.0%) were younger than 10 years old, 7 subjects (30.4%) were in the 11 to 20 years old group, 7 subjects (23.3%) were in the 21 to 30 years old group, 6 subjects (25%) were in the 31 to 40 years old, and 11 subjects (32.4%) were over 41 years old (**Figure 4D**). In 32 subjects with 1:32 AAV9 NAb titer, 8 subjects (18.6%) were younger than 10 years old, 4 subjects (17.4%) were in the 11 to 20 years old group, 6 subjects (20.0%) were in the 21 to 30 years old group, 4 subjects (16.7%) were in the 31 to 40 years old, and 10 subjects (29.4%) were over 41 years old (**Figure 4D**). In 15 subjects with 1:32 AAV843 NAb titer, 5 subjects (11.6%) were younger than 10 years old, 2 subjects (8.7%) were in the 11 to 20 years old group, 4 subjects (13.3%) were in the 21 to 30 years old group, 0 subjects (0%) were 31 to 40 years old, and 4 subjects (11.8%) were over 41 years old (**Figure 4D**).

The proportion of subjects with high AAV8 or AAV9 NAb titers over 41 years old was significantly higher than that in the less than 10 years old group ($P<0.05$). The AAV843 neutralizing

antibody titer was not significantly correlated with age.

Discussion

Recombined AAV technology has gradually grown as a key method for gene therapy. It can be used to treat many hereditary and acquired diseases. However, the immune response to wild-type AAV limits the efficient gene transduction of AAV in humans. Before treatment with rAAV, the AAV NAbs selection in patients is key to pharmacodynamic effects of gene therapy. The current study investigated the prevalence of neutralizing antibodies against AAV8, AAV9, and AAV843 in a Chinese population. Prevalence rates of AAV neutralizing antibodies in the study provide a theoretical basis for the development and clinical application of gene therapy drugs. Previously, prevalence of AAV8 NAb was 15-30% and the prevalence of AAV9 NAb was 33.5%, much lower than that of AAV2 (50-60%) and AAV1 (50.5%) NAbs in Europeans [12, 17]. In addition, prevalence rates of AAV8 and AAV9 NAbs in Japanese were 32.9% and 36.5%, respectively [24]. Therefore, AAV8 and AAV9 were selected as natural AAV serotypes for gene therapy. In this study, data shows that the prevalence of AAV8 and AAV9 NAbs reached 37.7% and 36.8%. However, AAV843 was only 25%. The titer in Chinese AAV8 and AAV9 NAbs showed no significant differences with the Japanese, but slightly higher than Europeans and Americans. This suggested that the prevalence of AAV8 and 9 NAbs was similar in Asian people. It has been reported that the prevalence of AAV1 NAb (69.8%) in Anhui and Beijing provinces in China was significantly higher than in Europe and America [25]. Prevalence of AAV antibodies in developing countries is higher than that in developed countries. This might be due to different living conditions, population density, sanitary conditions, and major histocompatibility complex (MHC) backgrounds in different countries [20]. Moore and his colleagues confirmed that the level of AAV NAbs might reflect the strength of an individual's immunity. Moreover, the individual's immunity may be related to heredity, leading to differences in AAV NAbs between different regions [26].

The number of all three AAV NAbs positive subjects was only 16.3% in young people, while it was 35.3% in older detected people ($P<0.001$). The positive rate of NAbs and titer both increased with age. A previous study showed that older subjects (≥41 years old) had a higher prevalence of multiple AAV Nabs, compared to younger subjects. Other studies have confirmed that the prevalence of AAV2 NAb was almost 30% in adults, but 4-15% in children [27]. In addition, a four-year continuous measurement for AAV2, AAV5, and AAV8 NAbs in children with hemophilia also confirmed that the positive rate of AAV NAbs increased in all children with advancing age [21]. These results were consistent with present results obtained in AAV8 and AAV9 NAbs assays.

Pre-existing AAV NAbs impaired the efficacy of AAV vector-mediated gene transfer by intravenous injections. Although there were lots of AAV serotypes, NAbs might make cross-reactivity with multiple AAV serotypes because of similar AAV capsid. Studies in humans have shown that various levels of NAbs are cross-reactive with AAV2 and other serotypes [12, 28]. Cross-reactivity of AAV NAbs could inhibit infections *in vivo* after injections [27]. Li and his colleagues showed that AAV5 and AAV8 NAbs were rarely observed in the absence of AAV2 infections, while AAV5 and AAV8 NAbs were commonly detected after AAV2 exposure [21]. Jun Mimuro and his colleagues showed that AAV2 could stimulate the cross-reactivity of AAV8 NAb and high titers of AAV2 NAb increased neutralization of AAV8 by NAb. In this experiment, there was a clear cross-reactivity among AAV8, AAV9, and AAV843. When subjects with NAb to AAV8 or AAV9 had co-prevalent NAbs to AAV843, the titer of NAb to AAV843 was lower than that to AAV8 and AAV9. AAV8 and AAV9 could boost AAV843 titers, further aggravating cross-reactivity.

Approximately 95% of people are naturally exposed to AAV. About half of them produce neutralizing antibodies. According to worldwide epidemiological statistics, the prevalence of AAV2 neutralizing antibody is approximately 50%, which prevents gene therapy based on AAV2 capsid. To evade AAV2 neutralizing antibody-mediated-AAV vector clearance, many strategies have been investigated to engineer AAV capsids, attempting to evade the recognition of NAbs. AAV could be coated with a polymer, such as polyethylene glycol or exosomes, to enhance gene transfer of the AAV vector, avoiding pre-existing humoral immunity [29-32]. It

was also a strategy to rationally mutate AAV to eliminate antibody recognition sites and to obtain new AAV capsid variants. Several clinical approaches are available for depleted neutralizing antibodies, including plasmapheresis, infusion of anti-CD20 (rituximab), and simultaneous delivery of excess empty AAV capsid as bait [18, 33].

In conclusion, the seropositivity of AAV8 NAb, AAV9 Nab, and AAV843 was low in the Chinese population. AAV843 was significantly less immunogenic than the other two serotypes. All three AAV serotypes can be used as candidate serotypes for gene therapy in Chinese populations, but AAV843 is the most optimal. The current study research provides important information for clinical gene therapy using AAV vectors for the Chinese population.

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Disclosure of conflict of interest

None.

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Table S1. Neutralizing antibody seropositivity against AAV serotypes 8, 9, and 843 sub-grouped by ages for the Chinese

Age (years)	AAV neutralizing antibodies seropositivity, num (%)		
	AAV8	AAV9	AAV843
≤10	8 (18.6%)	9 (20.9%)	7 (16.3%)
11-20	9 (39.1%)	7 (30.4%)	5 (21.7%)
21-30	13 (43.3%)	10 (33.3%)	8 (26.7%)
31-40	12 (50.0%)	12 (50.0%)	6 (25.0%)
≥41	17 (50.0%)	17 (50.0%)	13 (38.2%)
Total	59	55	39

Table S2. Neutralizing antibodies against multiple AAV serotypes sub-grouped by ages for the Chinese

Age (years)	AAV neutralizing antibodies seropositivity, num (%)			
	Sero-negative for 3 AAV serotypes	Sero-positive for 3 AAV serotypes	Sero-positive for 2 AAV serotypes	Sero-positive for 1 AAV serotype
≤10	34 (37.4%)	7 (18.9%)	1 (6.3%)	1 (10.0%)
11-20	14 (15.4%)	5 (13.5%)	2 (12.5%)	2 (20.0%)
21-30	17 (18.7%)	7 (18.9%)	4 (25.0%)	2 (20.0%)
31-40	11 (12.1%)	6 (16.2%)	5 (31.3%)	2 (20.0%)
≥41	15 (16.5%)	12 (32.4%)	4 (25.0%)	3 (30.0%)
Total	91	37	16	10

Table S3. Neutralizing antibody seropositivity against AAV serotypes 8, 9, and 843 in subjects in each age group

AAV serotype	AAV neutralizing antibodies seropositivity, num (%)				
	≤10	11-20	21-30	31-40	≥41
AAV8	8 (13.6%)	9 (15.3%)	13 (22.0%)	12 (20.3%)	17 (28.8%)
AAV9	9 (16.4%)	7 (12.7%)	10 (18.2%)	12 (21.8%)	17 (30.9%)
AAV843	7 (17.9%)	5 (12.8%)	8 (20.5%)	6 (15.4%)	13 (33.3%)

Table S4. Neutralizing antibody seropositivity multiple AAV serotypes in subjects in the same age group

	AAV neutralizing antibodies seropositivity, num (%)				
	≤10	11-20	21-30	31-40	≥41
Sero-negative for 3 AAV serotypes	34 (79.1%)	14 (60.9%)	17 (56.7%)	11 (45.8%)	15 (44.1%)
Sero-positive for 3 AAV serotypes	7 (16.3%)	5 (21.7%)	7 (23.3%)	6 (25.0%)	12 (35.3%)
Sero-positive for 2 AAV serotypes	1 (2.3%)	2 (8.7%)	4 (13.3%)	5 (20.8%)	4 (11.8%)
Sero-positive for 1 AAV serotype	1 (2.3%)	2 (8.7%)	2 (6.7%)	2 (8.3%)	3 (8.8%)
Total	43	23	30	24	34